

*Fig. 1*

## NR-LU-13 Heavy chain variable region sequences

GAG GTT CAG CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA GTC AGG TTG TCC TGC Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys	22
<u>CDR1</u>	
ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT ATG CAC TGG GTG ATA GAG AGG CCT GAA CAG GGC Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr Met His Trp Val Ile Glu Arg Pro Glu Gln Gly	44
<u>CDR2</u>	
CTG GAG TGG ATT GGA AGG ATT GAT CCT GCG AAT GGT AAT ACT AAA TGT GAC CCG AAG TTC CAG GGC Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Cys Asp Pro Lys Phe Gln Gly	66
AAG GCC ACT ATA ACA GCA GAC ACA TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser	88
<u>CDR3</u>	
GAG GAC ACT GCC GTC TAT TAC TGT TCT AGA GAG GTC CTA ACT GGG ACG TGG TCT TTG GAC TAC TGG Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg Glu Val Leu Thr Gly Thr Trp Ser Leu Asp Tyr Trp	110
GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA Gly Gln Gly Thr Ser Val Thr Val Ser Ser	120

## NR-LU-13 Light chain variable region sequences

GAC ATC CAG ATG ATT CAG TCT CCA TCG TCC ATG TTT GCC TCT CTG GGA GAC AGA GTC AGT CTC TCT Asp Ile Gln Met Ile Gln Ser Pro Ser Ser Met Phe Ala Ser Leu Gly Asp Arg Val Ser Leu Ser	22
<u>CDR1</u>	
TGT CGG GCT AGT CAG GGC ATT AGA GGT AAT TTA GAC TGG TAT CAG CAG AAA CCA GGT GGA ACT ATT Cys Arg Ala Ser Gln Gly Ile Arg Gly Asn Leu Asp Trp Tyr Gln Gln Lys Pro Gly Gly Thr Ile	44
<u>CDR2</u>	
AAA CTC CTG ATC TAC TCC ACA TCC AAT TTA AAT TCT GGT GTC CCA TCA AGG TTC AGT GGC AGT GGC Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Asn Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly	66
TCT GGG TCA GAT TAT TCT CTC ACC ATC AGC AGC CTA GAC TCT GAA GAT TTT GCA GAC TAT TAC TGT Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Asp Ser Glu Asp Phe Ala Asp Tyr Tyr Cys	88
<u>CDR3</u>	
CTA CAG CGT AAT GCG TAT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA Leu Gln Arg Asn Ala Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	107

*Fig. 2*

Light Chain

1		5		10
ASP	ILE	GLN	MET	THR
				GLN
				SER
				PRO
				SER
				SER
11		15		20
LEU	SER	ALA	SER	VAL
				GLY
				ASP
				ARG
				VAL
				THR
21		25		30
ILE	THR	CYS	ARG	ALA
				SER
				GLN
				GLY
				ILE
				ARG
31		35		40
GLY	ASN	LEU	ASP	TRP
				TYR
				GLN
				GLN
				LYS
				PRO
41		45		50
GLY	LYS	GLY	PRO	LYS
				LEU
				LEU
				ILE
				TYR
				SER
51		55		60
THR	SER	ASN	LEU	ASN
				SER
				GLY
				VAL
				PRO
				SER
61		65		70
ARG	PHE	SER	GLY	SER
				GLY
				SER
				GLY
				SER
				ASP
71		75		80
TYR	THR	LEU	THR	ILE
				SER
				SER
				LEU
				GLN
				PRO
81		85		90
GLU	ASP	PHE	ALA	THR
				TYR
				TYR
				CYS
				LEU
				GLN
91		95		100
ARG	ASN	ALA	TYR	PRO
				TYR
				THR
				PHE
				GLY
				GLN
101		105		
GLY	THR	LYS	LEU	GLU
				ILE
				LYS

The humanized sequence of NRX451 light chain, residue positions which differ between NR-LU-13 and NRX451-humanized are marked with bold type.

*Fig. 3*

Heavy Chain

1		5		10
GLN	VAL	GLN	LEU	VAL
		GLN	SER	GLY
			ALA	GLU
11		15		20
VAL	LYS	LYS	PRO	GLY
		ALA	SER	VAL
			LYS	VAL
21		25		30
SER	CYS	LYS	ALA	SER
		GLY	PHE	ASN
			ILE	LYS
31		35		40
ASP	THR	TYR	MET	HIS
		TRP	VAL	ARG
			GLN	ALA
41		45		50
PRO	GLY	GLN	GLY	LEU
		GLN	TRP	MET
			GLY	ARG
51		55		60
ILE	ASP	PRO	ALA	ASN
		GLY	ASN	THR
			LYS	CYS
61		65		70
ASP	LEU	SER	PHE	GLN
		GLY	ARG	VAL
			THR	ILE
71		75		80
THR	ALA	ASP	THR	SER
		ILE	ASN	THR
			ALA	TYR
81		85		90
MET	GLU	LEU	SER	SER
		LEU	ARG	SER
			ASP	ASP
91		95		100
THR	ALA	VAL	TYR	TYR
		CYS	SER	ARG
			GLU	VAL
101		105		110
LEU	THR	GLY	THR	TRP
		SER	LEU	ASP
			TYR	TRP
111		115		120
GLY	GLN	GLY	THR	LEU
		VAL	THR	VAL
			SER	SER

The humanized sequence of NRX451 light chain, residue positions which differ between NR-LU-13 and NRX451-humanized are marked with bold type.

*Fig. 4*

Alignment of the Light Chain Variable Regions of  
NR-LU-13 (top) and humanized NRX451 (bottom).

```
DIQMISSPSSMFASLGDRVSLSC RASQGIRGNLD WYQKPGGTIKLLIY STSNLNS
.....
DIQMTQSPSSLSASVGDRTITC RASQGIRGNLD WYQKPCKGPKLLIY STSNLNS
                        CDR1                      CDR2

GVPSRFSGSGSGSDYSLTISSLESEDFADYYC LQRAYPYTF GGGTKLEIK
.....
GVPSRFSGSGSGSDYTLTISSLQPEDFADYYC LQRAYPYTF GGGTKLEIK
                        CDR3
```

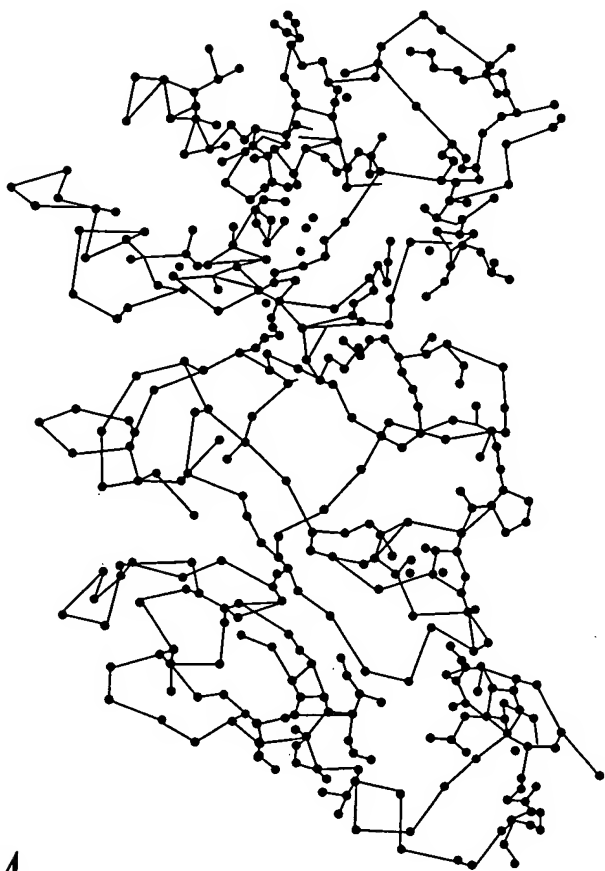
Alignment of the Heavy Chain Variable Regions of  
NR-LU-13 (top) and humanized NRX451 (bottom).

```
EVQLQQSGAELVKPGASVRLSCTASGFNIK DTYMH WVIERPEQGLEWIG
.....
QVQLVQSGAEVKKPGASVKVSCKASGFNIK DTYMH WVRQAPGQGLQWMG
                        CDR1

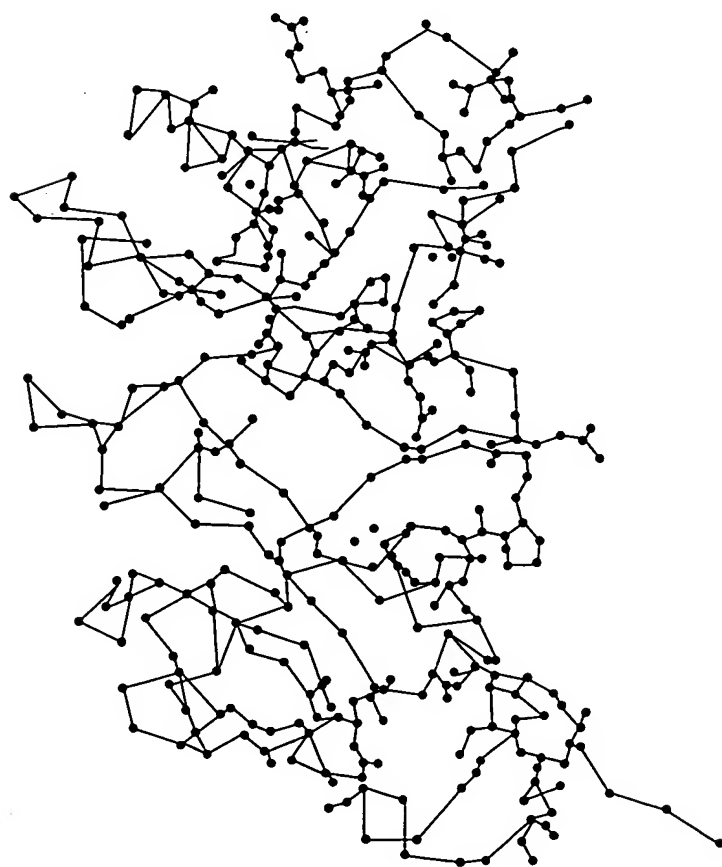
RIDPANGNTK CDPKFQGKATITADTSSNTAYLQLSSLTSEDAVYYCS
.....
RIDPANGNTK CDLSFQGRVTITADTSINTAYMELSSLRSDAVYYCS
                        CDR2

REVLGTWSLDY WGQGTSVTVSS
.....
REVLGTWSLDY WGQGTLVTVSS
                        CDR3
```

*Fig. 5*



*Fig. 6A*



*Fig. 6B*

Same frequencies, but matching with human sequences. Only one occurrence of Asp at position 182 is found and no occurrences of Cys at position 181.

RES	181	182
A	-	0.48
R	-	0.02
N	0.01	0.18
D	0.00	0.00
C	0.00	0.00
Q	0.00	-
E	-	-
G	0.00	0.01
H	0.00	-
I	-	0.00
L	-	0.00
K	0.00	0.00
M	-	-
F	0.03	-
P	0.00	0.01
S	0.01	0.23
T	-	0.02
W	0.00	-
Y	0.91	-
V	0.00	0.02
X	0.01	0.02
O	-	-
-	-	-
Z	-	-
B	-	0.00
Total	1.00	1.00

*Fig. 7A*

Sequence positions 50 and 183 are structural mutations within 5 A of the CDR's. Frequency of residue types at these positions are identical.

Position 50 (153 human lambda sequences)

RES	50
A	-
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	-
K	-
M	0.00
F	-
P	0.93
S	-
T	-
W	-
Y	-
V	-
X	0.06
O	-
-	-
Z	-
B	-
Total	1.00

*Fig. 7B*



Position 50 (279 human kappa sequences)

RES	50
A	0.00
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	0.00
K	-
M	-
F	-
P	0.96
S	-
T	-
W	-
Y	-
V	-
X	0.03
O	-
-	-
Z	-
B	-
Total	1.00

*Fig. 7C*

Position 50 is highly conserved in all the sequences, but proline can be exchanged by Ile or Leu. The framework used for the light chain (6fab) does have an Ile at this position. If this position is compared to other structures the backbone torsions are the same for structures with a Pro and an Ile at this position.

Position 50 (153 human lambda sequences)

RES	183
A	0.06
R	-
N	0.00
D	0.21
C	-
Q	0.15
E	0.01
G	0.01
H	-
I	0.00
L	0.00
K	0.00
M	-
F	0.00
P	0.40
S	0.01
T	0.01
W	-
Y	0.00
V	0.08
X	0.02
O	-
-	-
Z	-
B	0.00
Total	1.00

*Fig. 7D*

Position 183 (1210 mouse sequences)

RES	183
A	0.16
R	0.00
N	0.00
D	0.13
C	-
Q	0.16
E	0.25
G	0.02
H	0.00
I	-
L	-
K	0.00
M	-
F	-
P	0.17
S	0.08
T	0.00
W	-
Y	-
V	0.00
X	0.02
O	-
-	-
Z	-
B	-
Total	1.00

Leu is seen in human sequences at this position, but never in murine sequences, for both human and murine Sequences P is the most frequently occurring residue at position 183.

*Fig. 7E*

Comments for pcDNA3:  
5446 nucleotides

CMV promotor: bases 209-863  
T7 promotor: bases 864-882  
Polylinker: bases 889-994  
Sp6 promotor: bases 999-1016  
BGH poly A: bases 1018-1249  
SV40 promotor: bases 1790-2115  
SV40 origin of replication: bases 1984-2069  
Neomycin ORF: bases 2151-2945  
SV40 poly A: bases 3000-3372  
ColE1 origin: bases 3632-4305  
Ampicillin ORF: bases 4450-5310

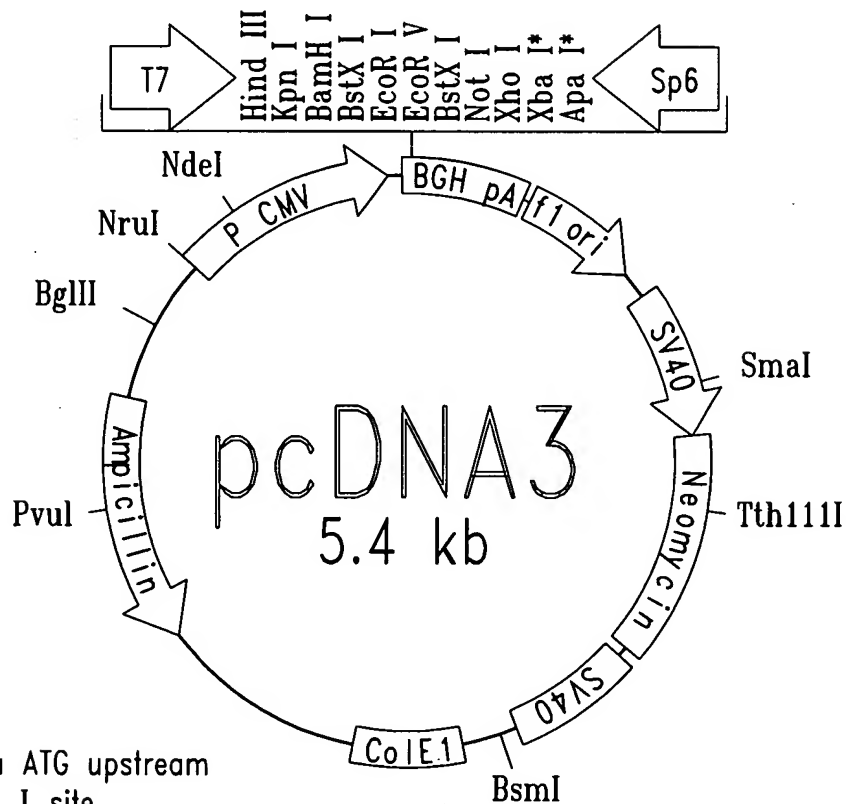
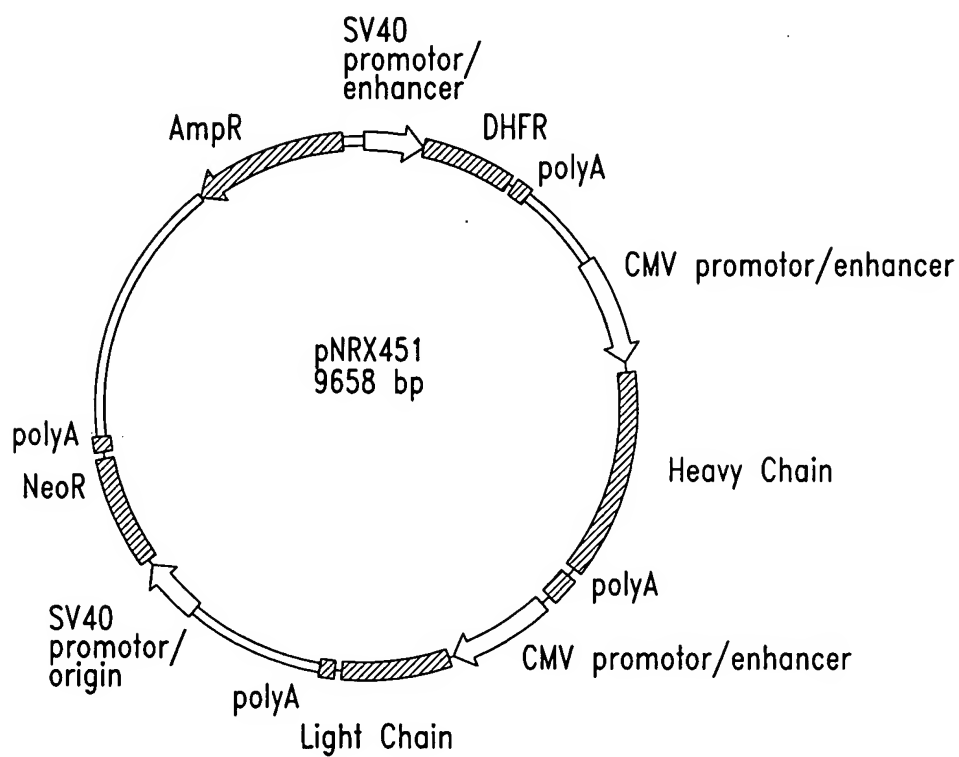
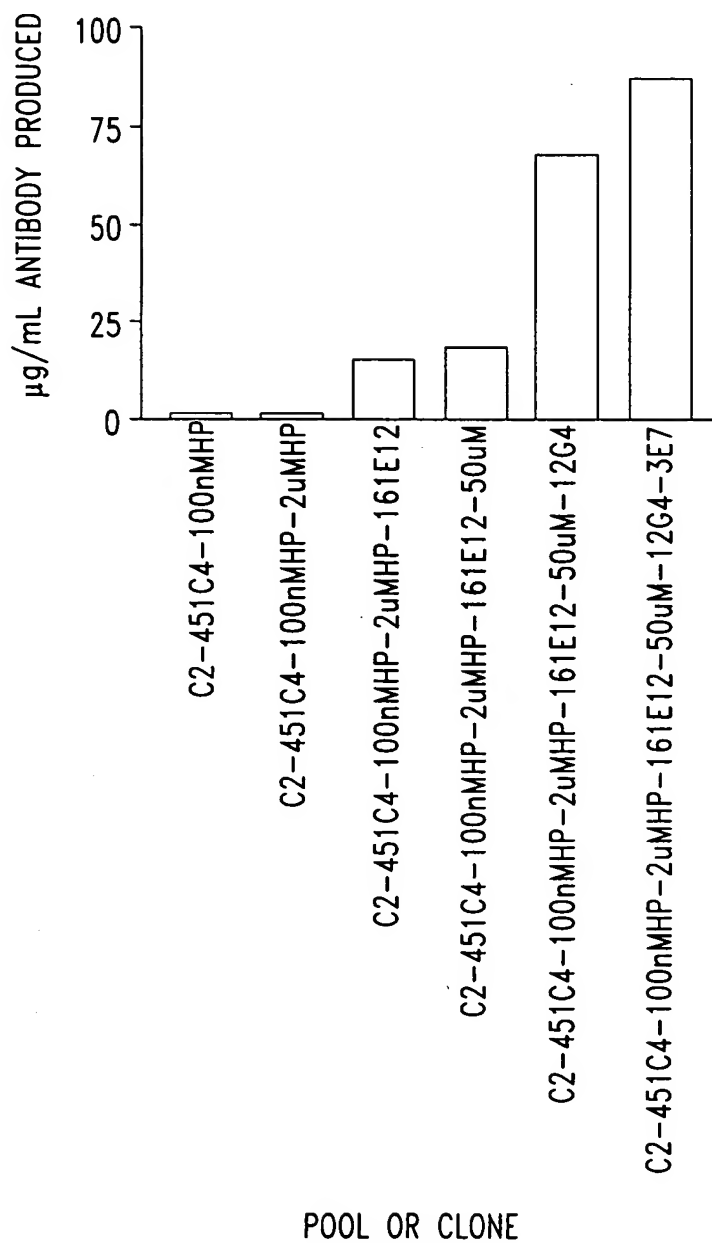


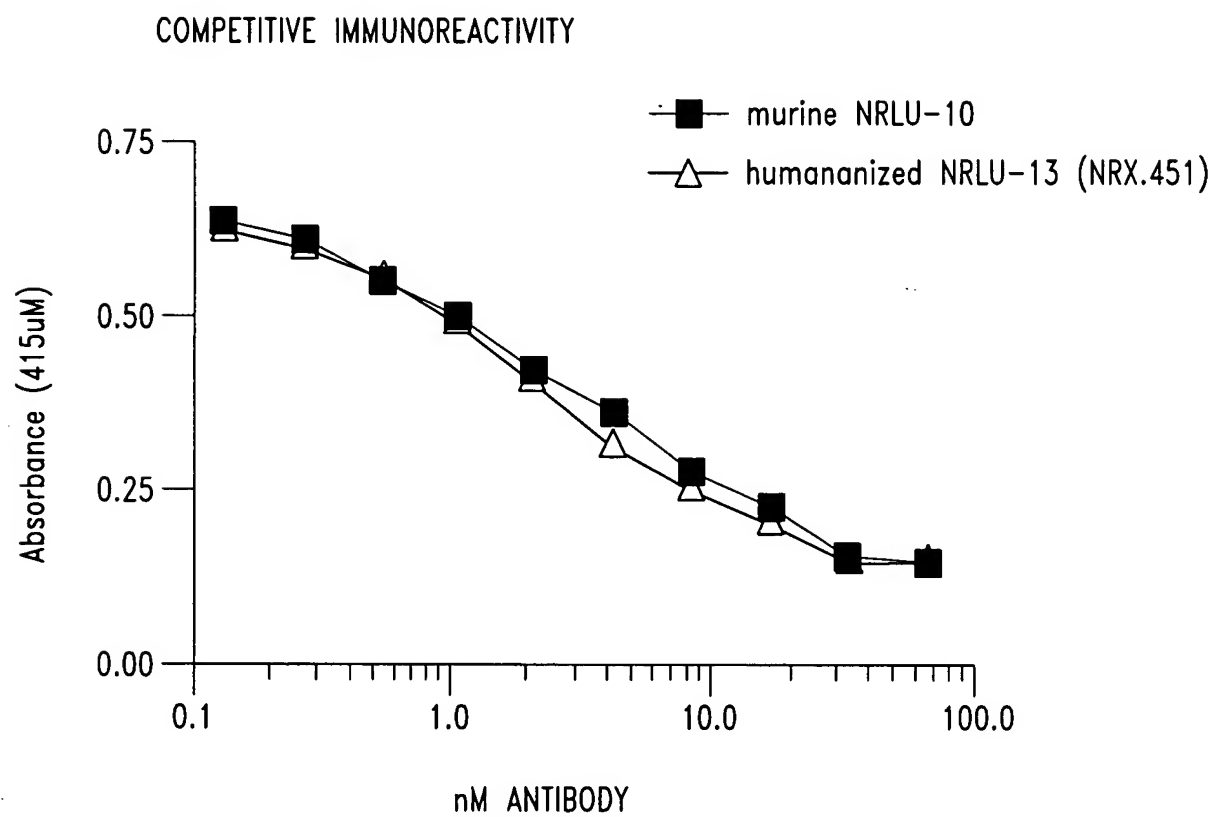
Fig. 8



*Fig. 9*



*Fig. 10*



*Fig. 11*

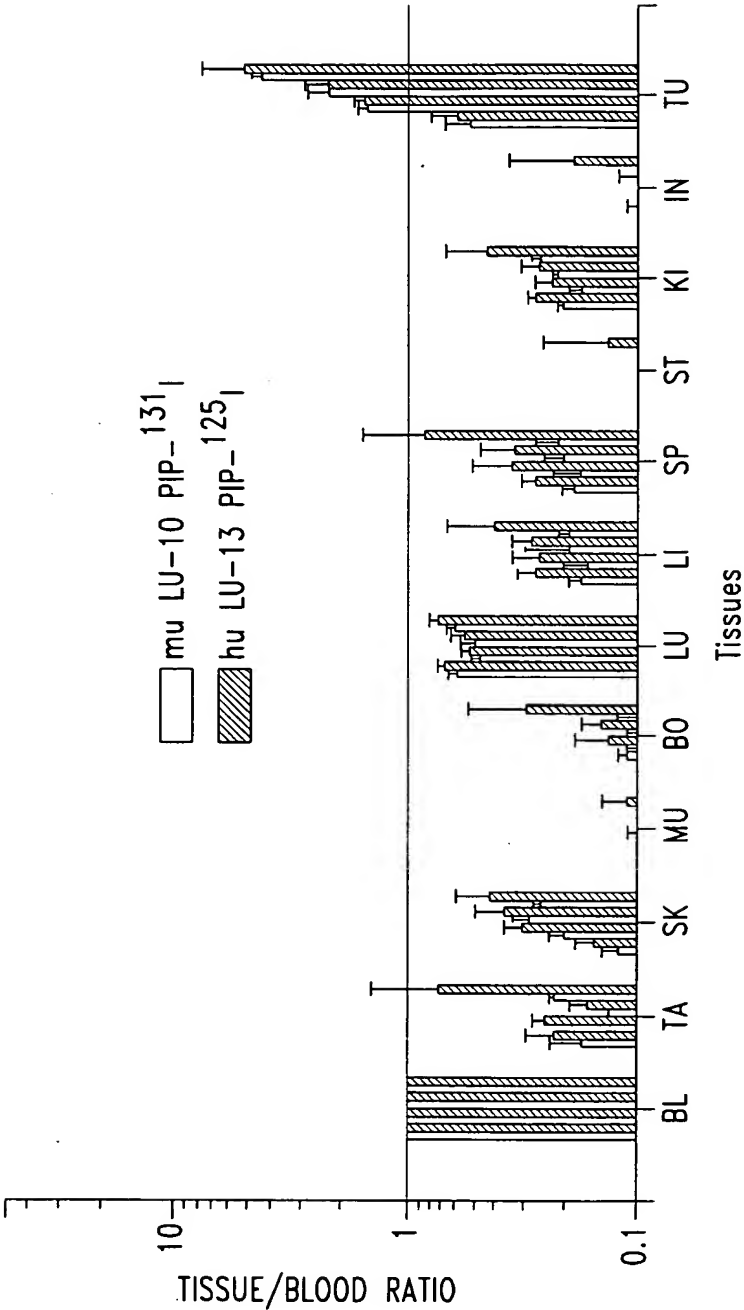


Fig. 12



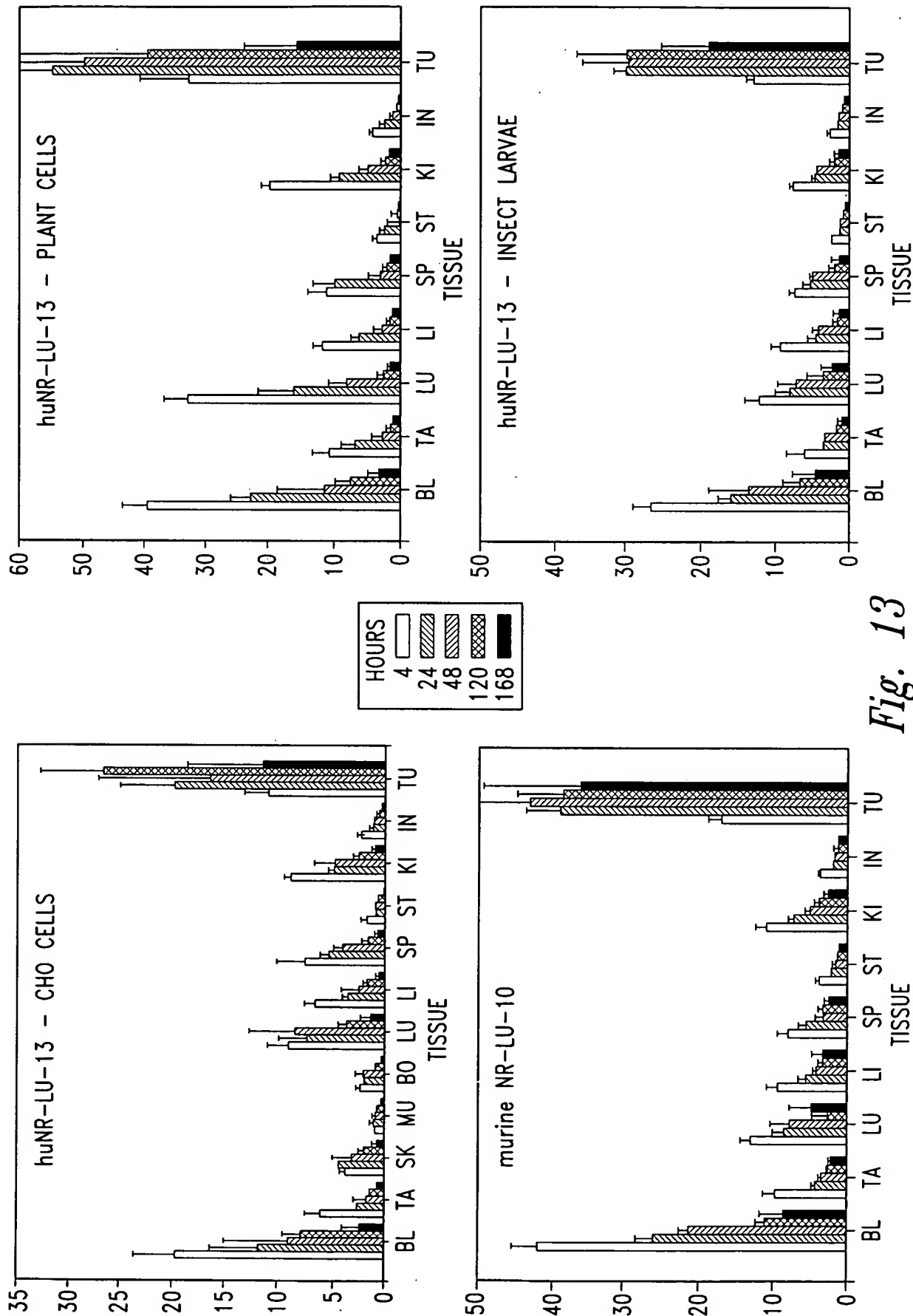


Fig. 13

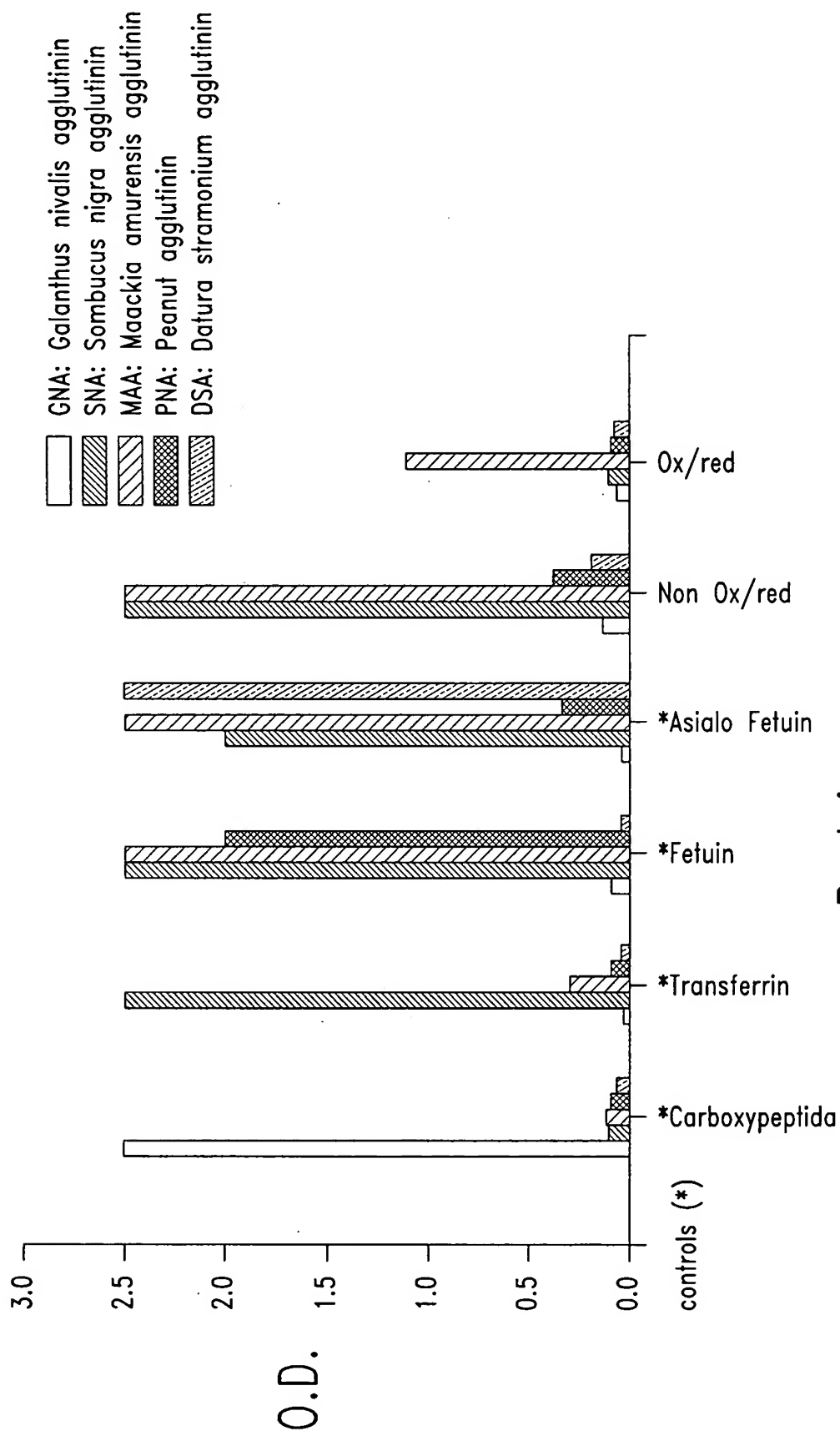
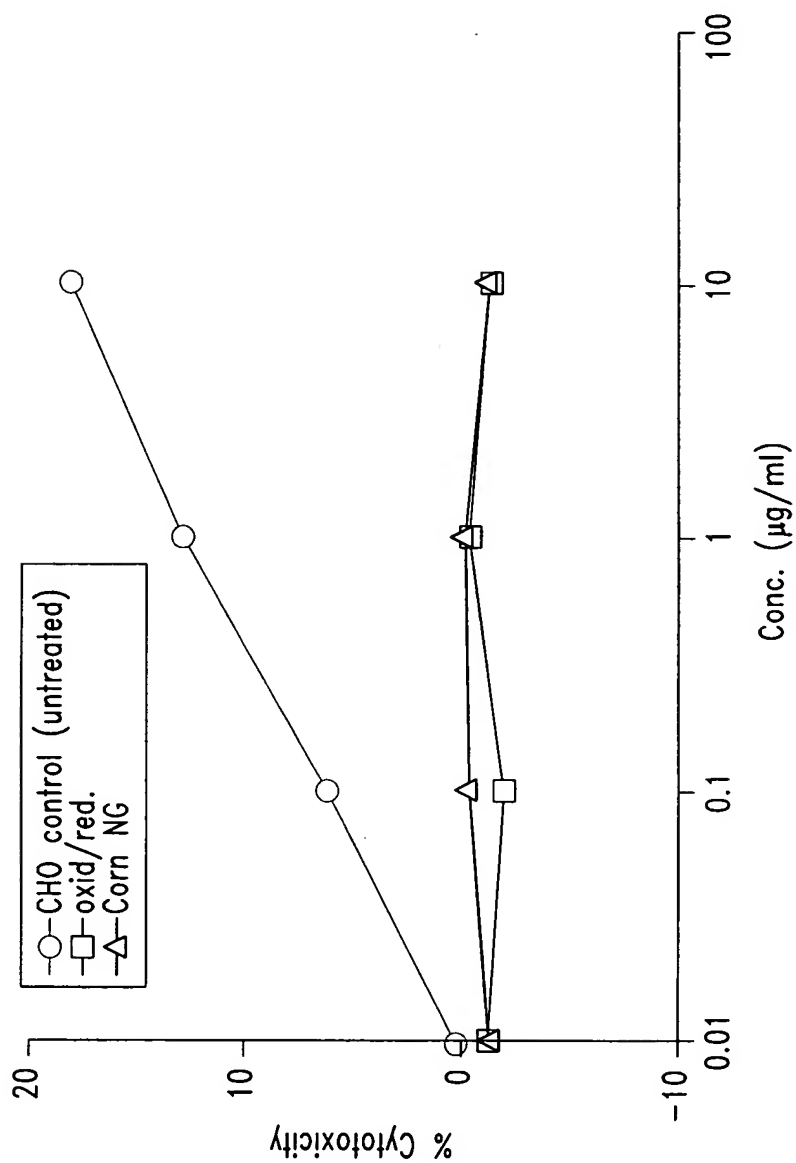
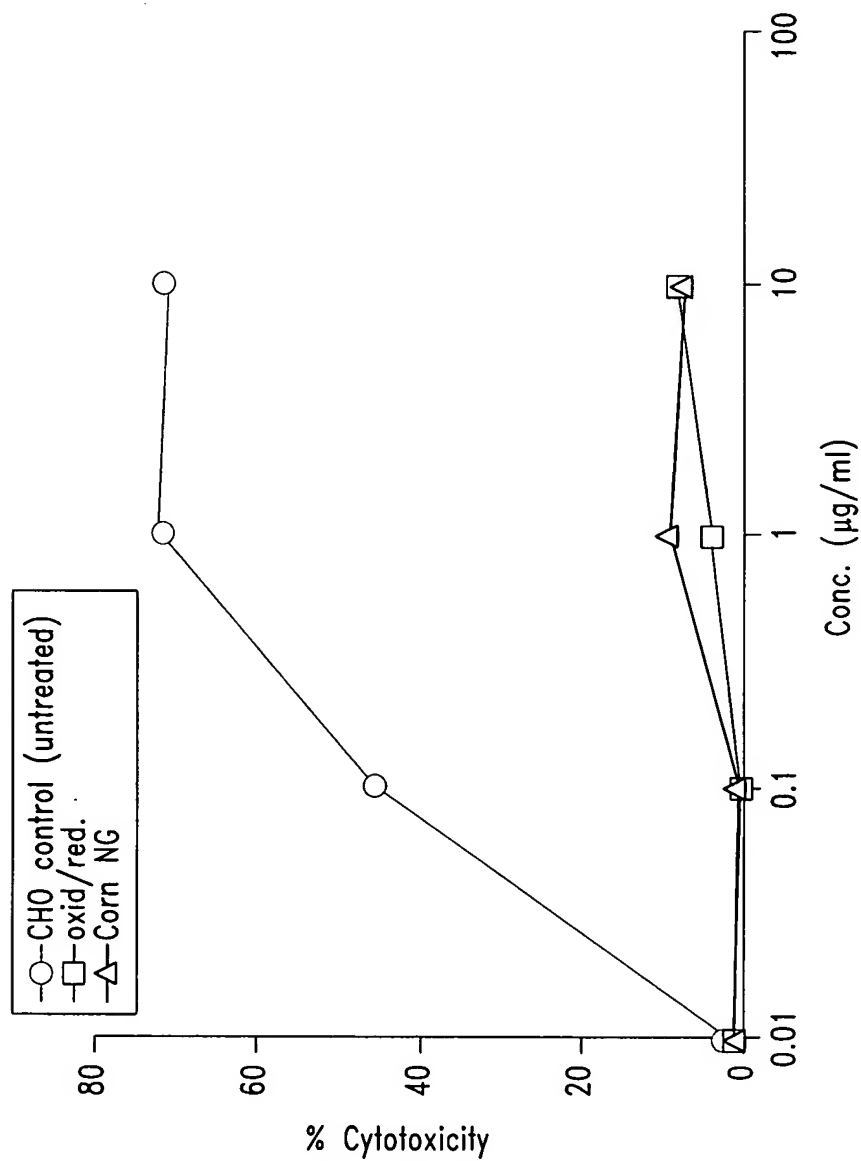


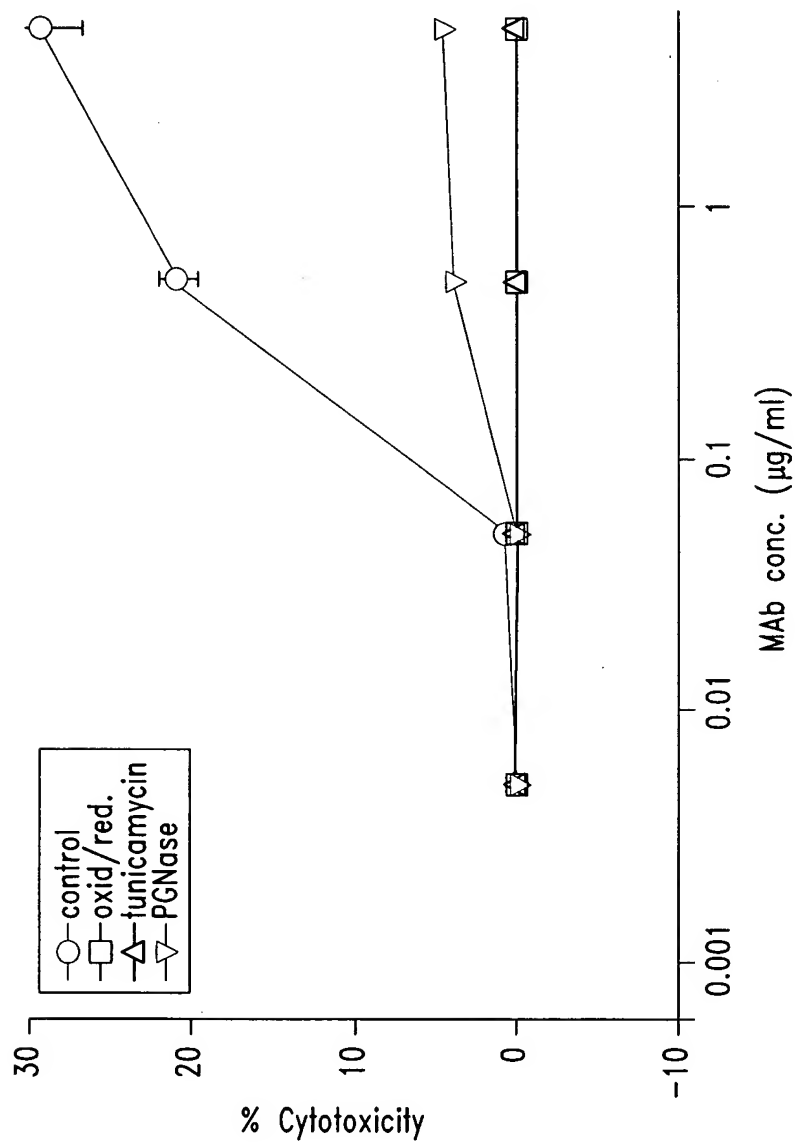
Fig. 14



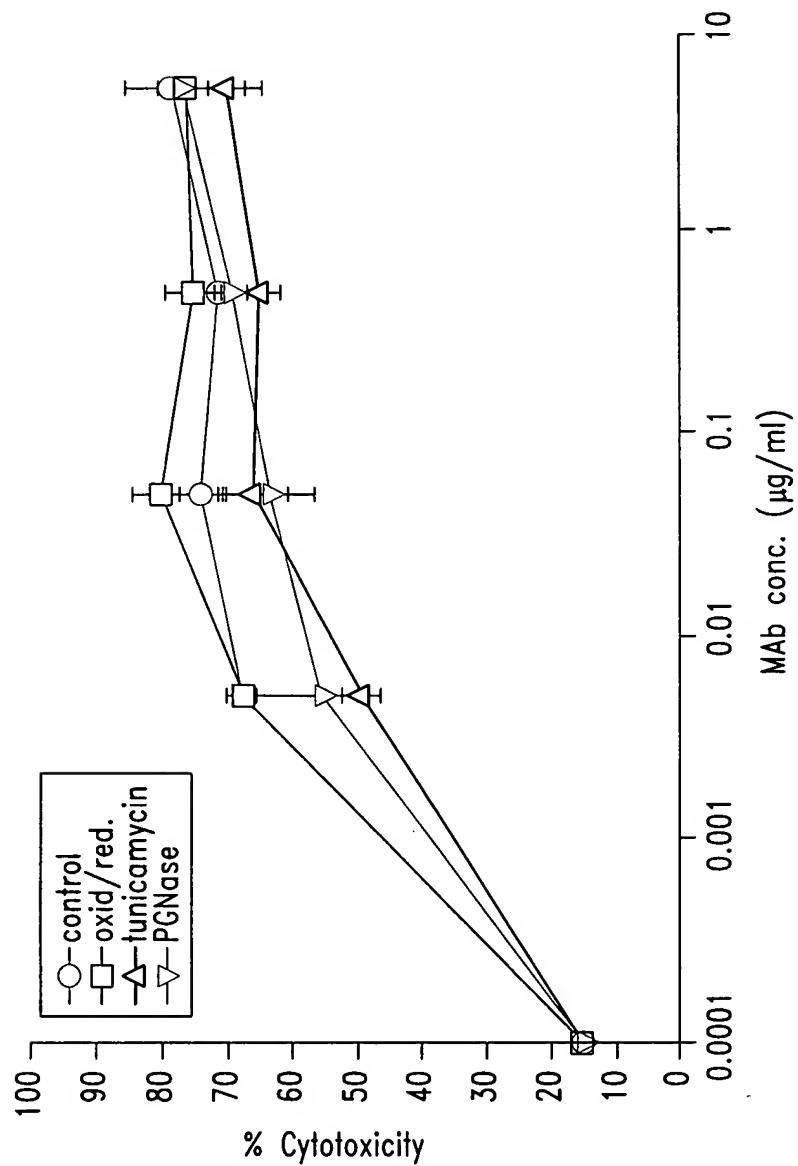
*Fig. 15A*



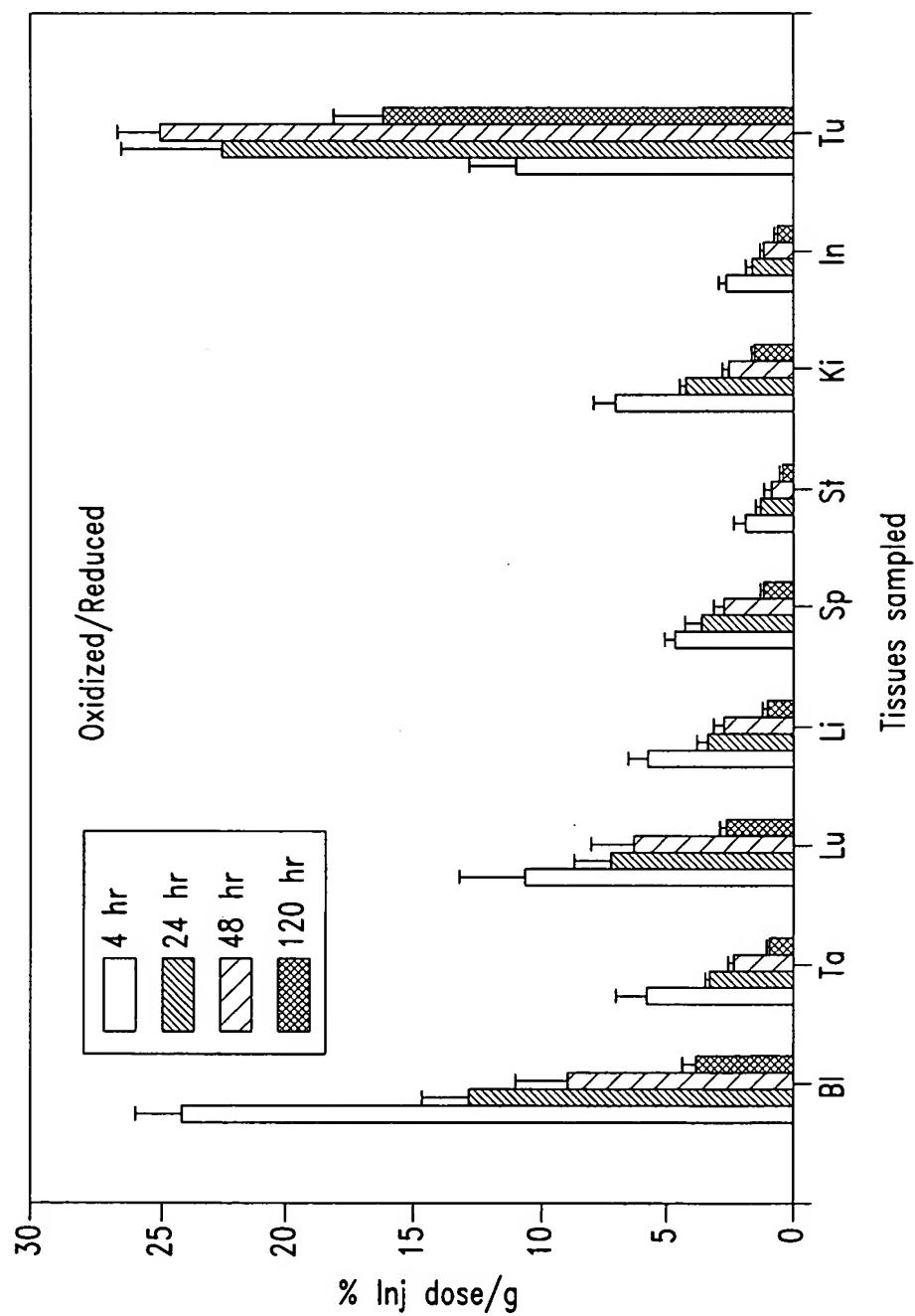
*Fig. 15B*



*Fig. 15C*



*Fig. 15D*



*Fig. 16A*

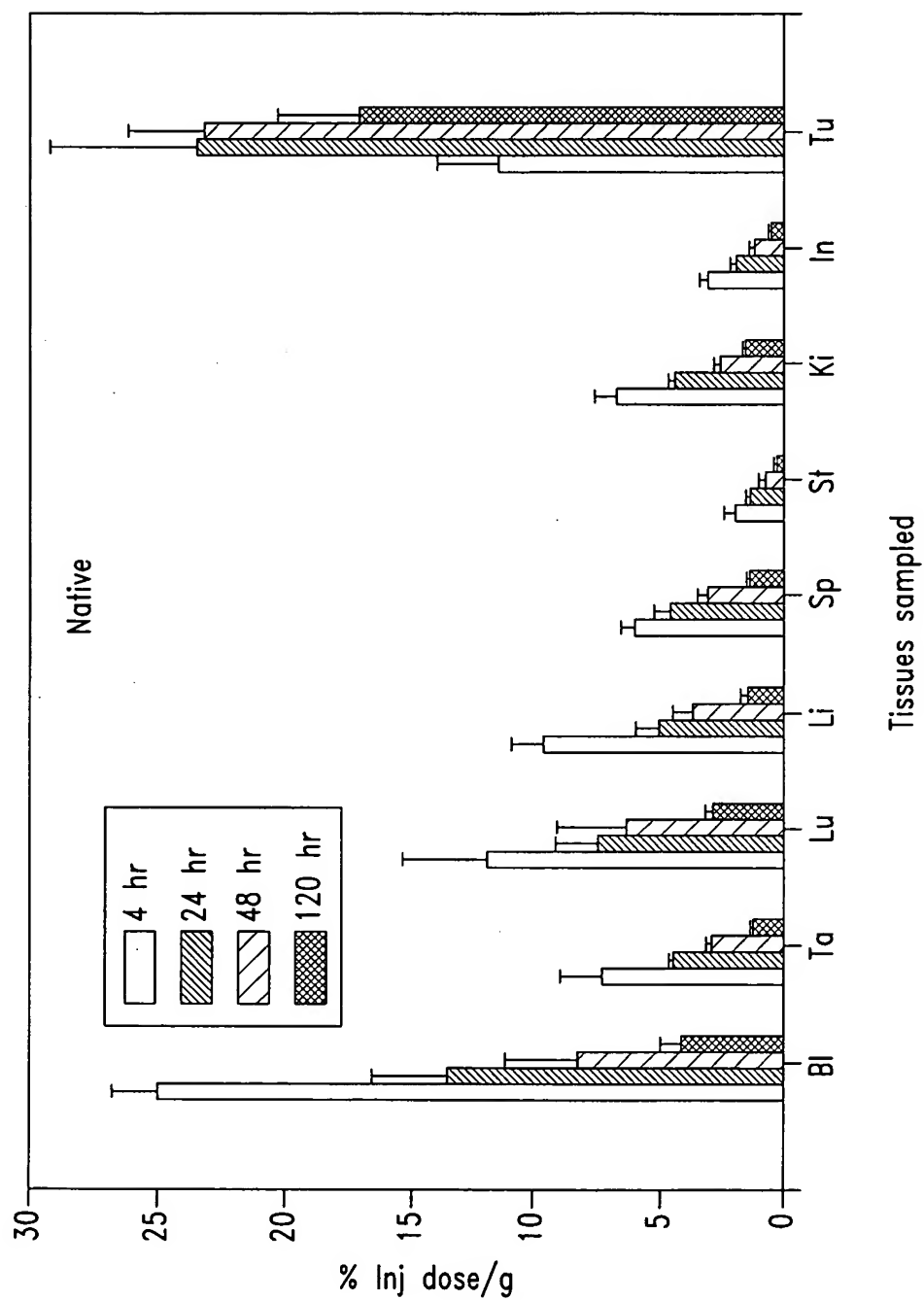


Fig. 16B



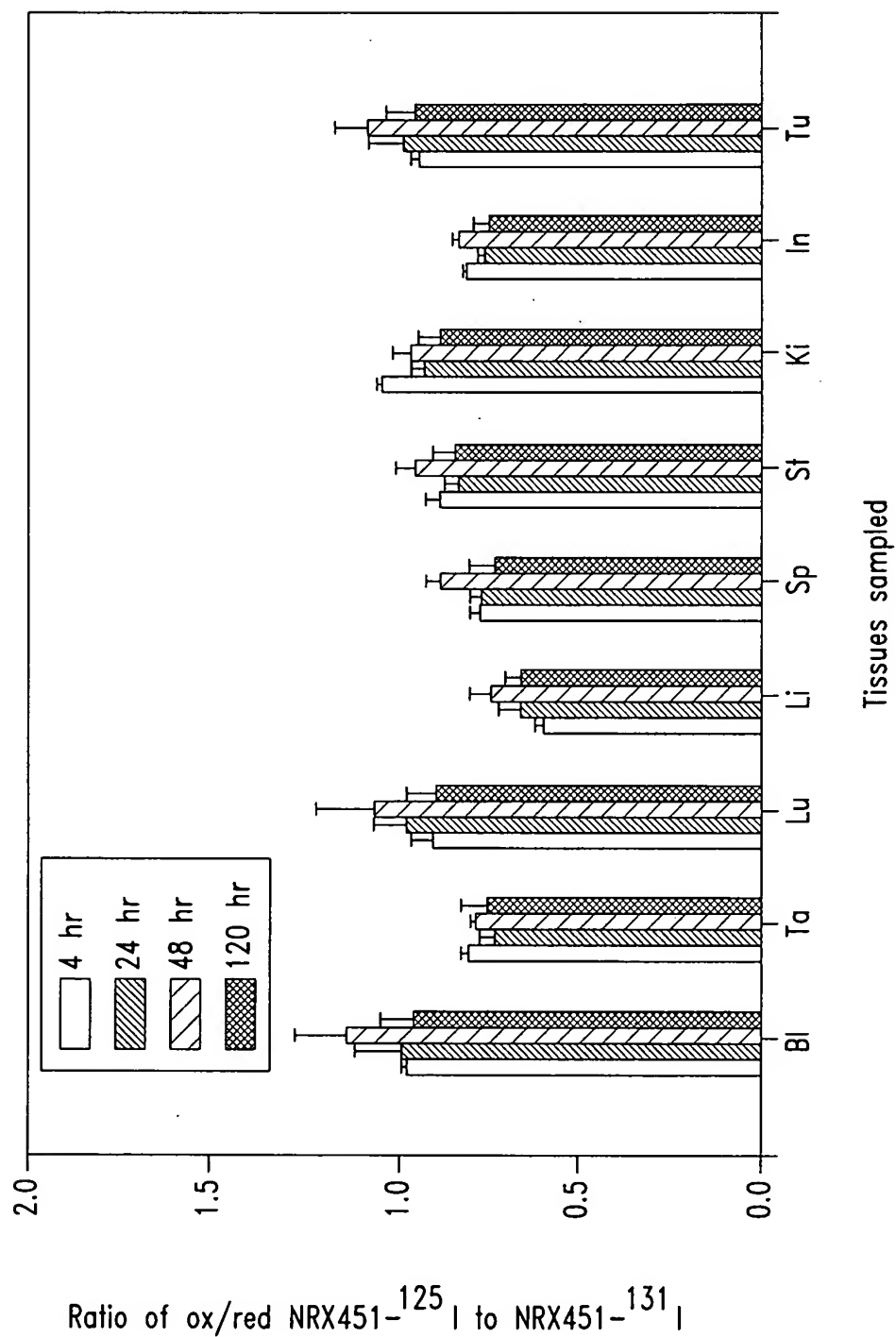


Fig. 16C

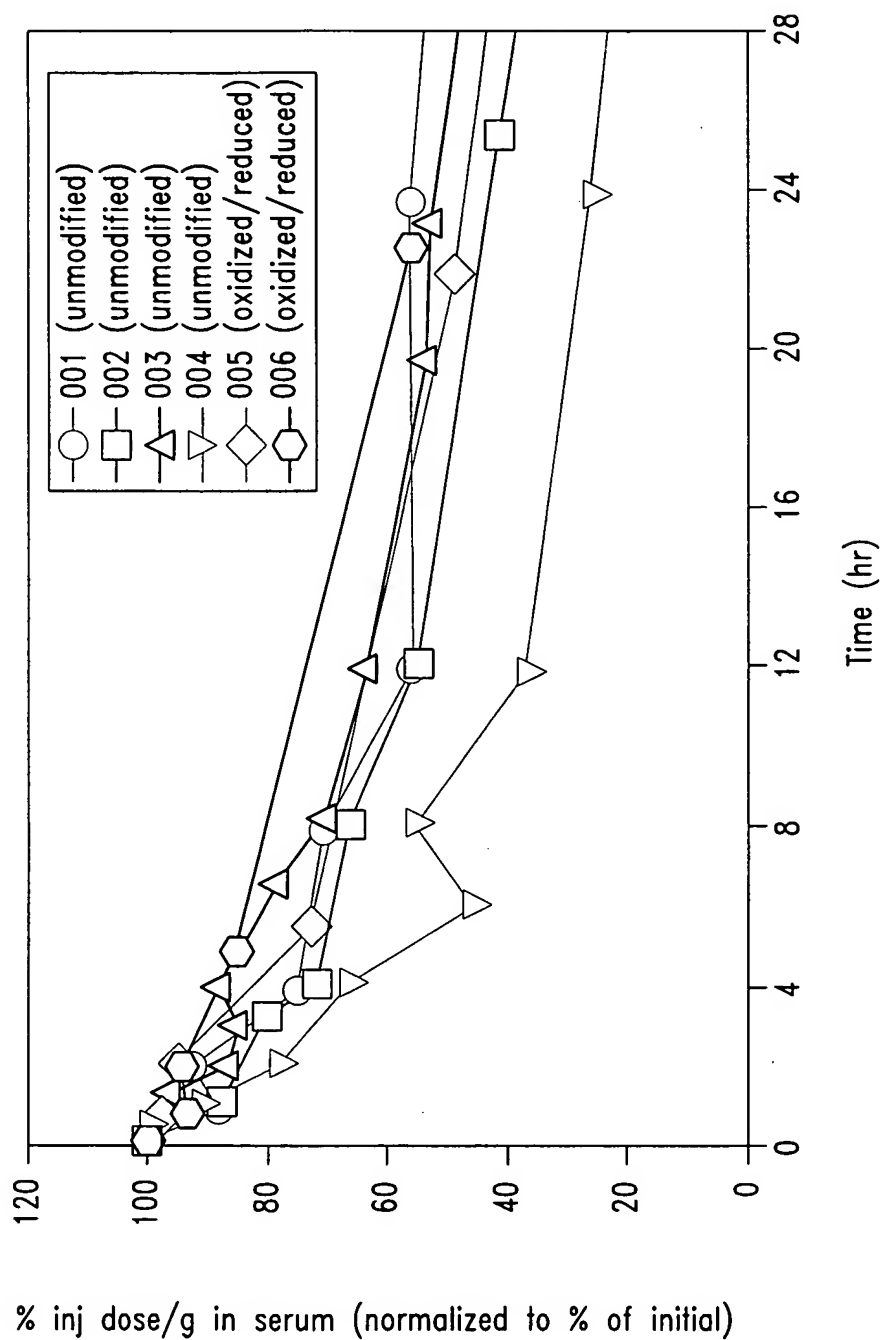
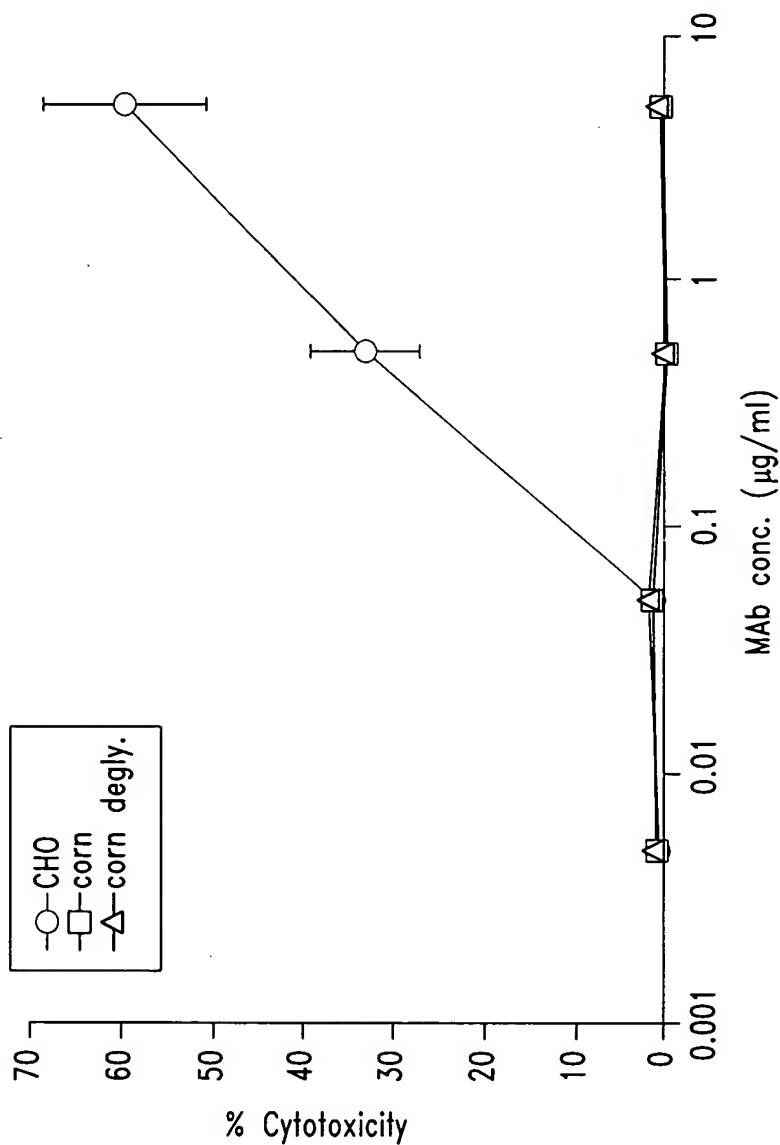
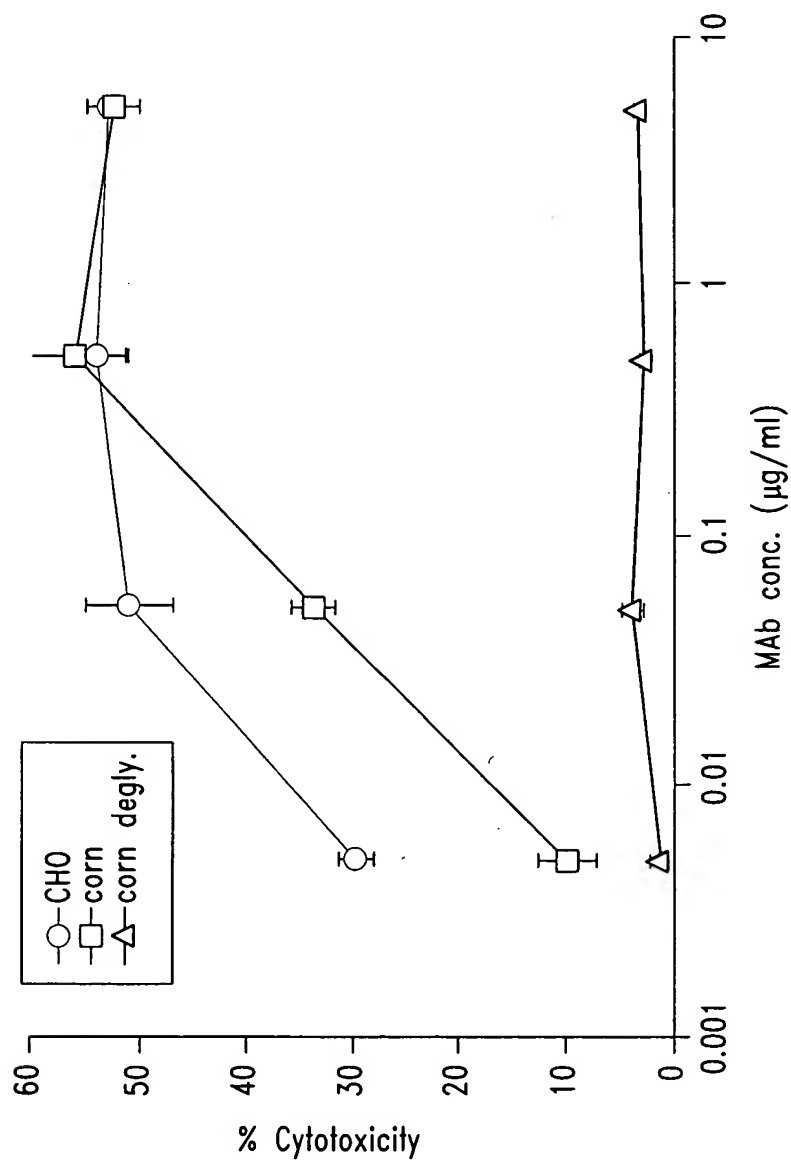


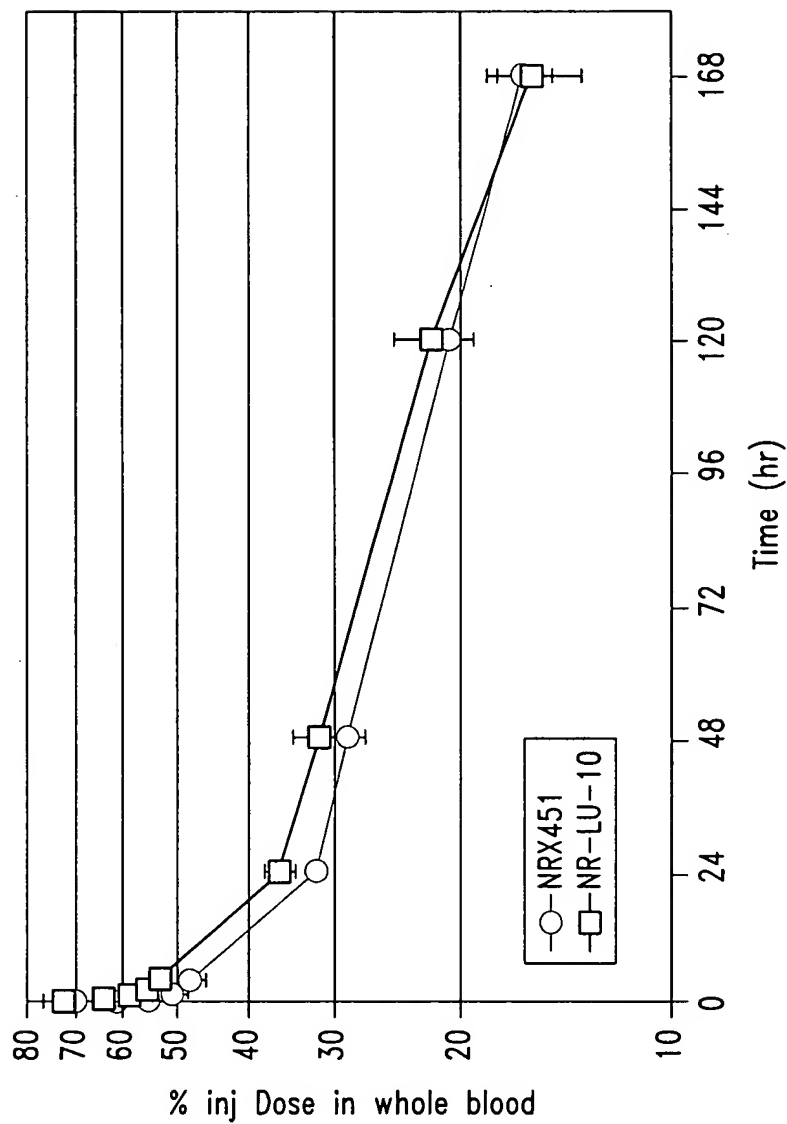
Fig. 17



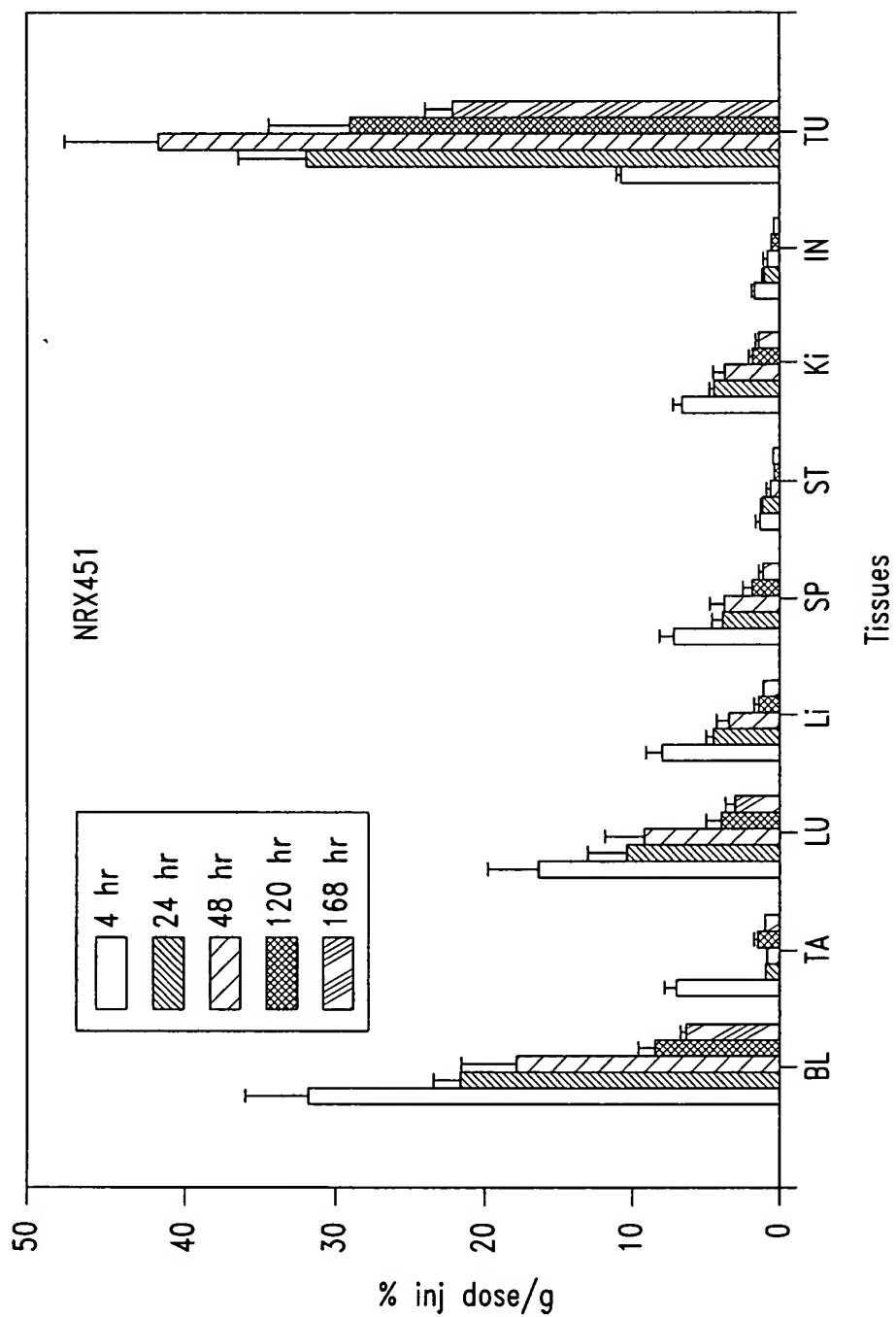
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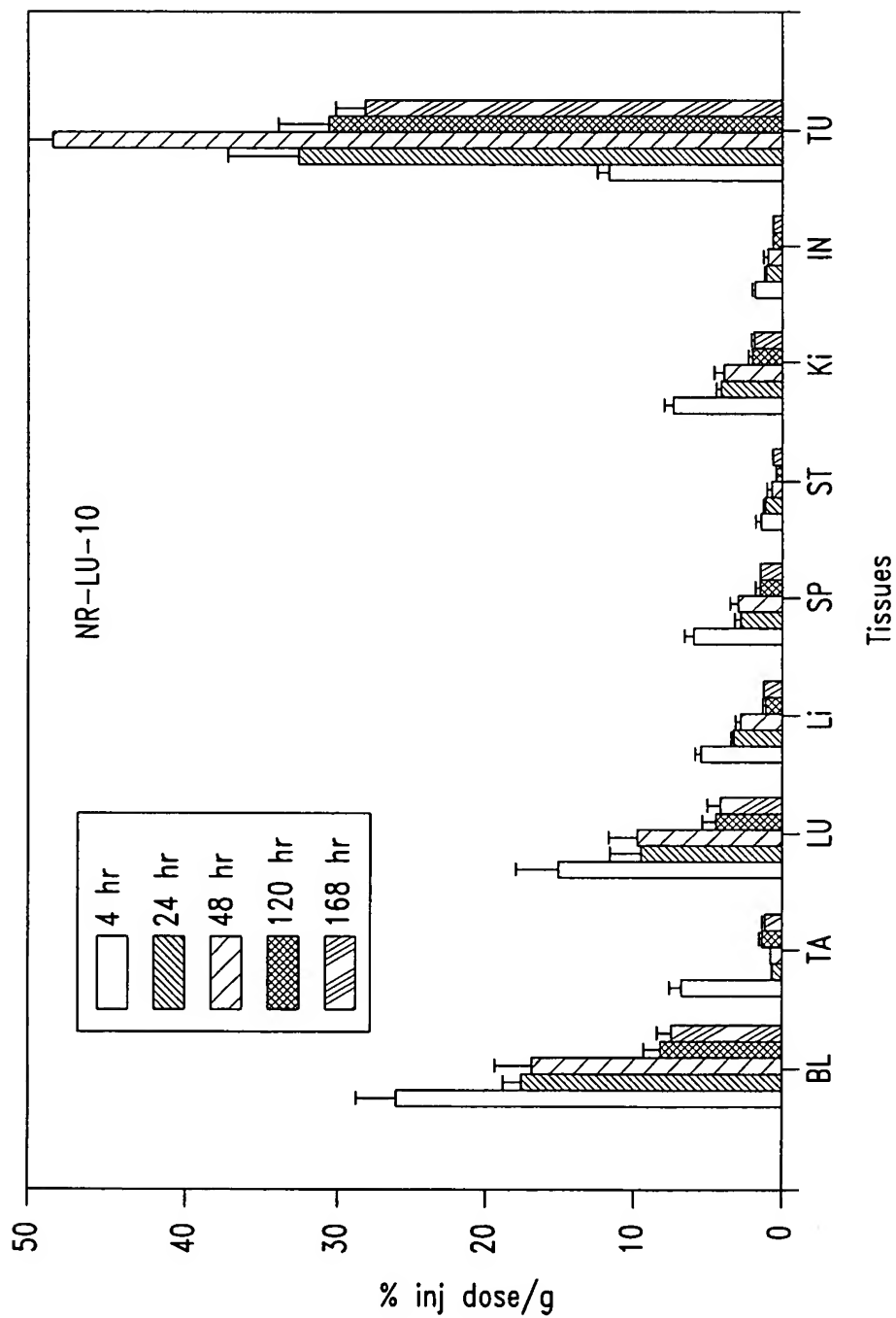
*Fig. 19*



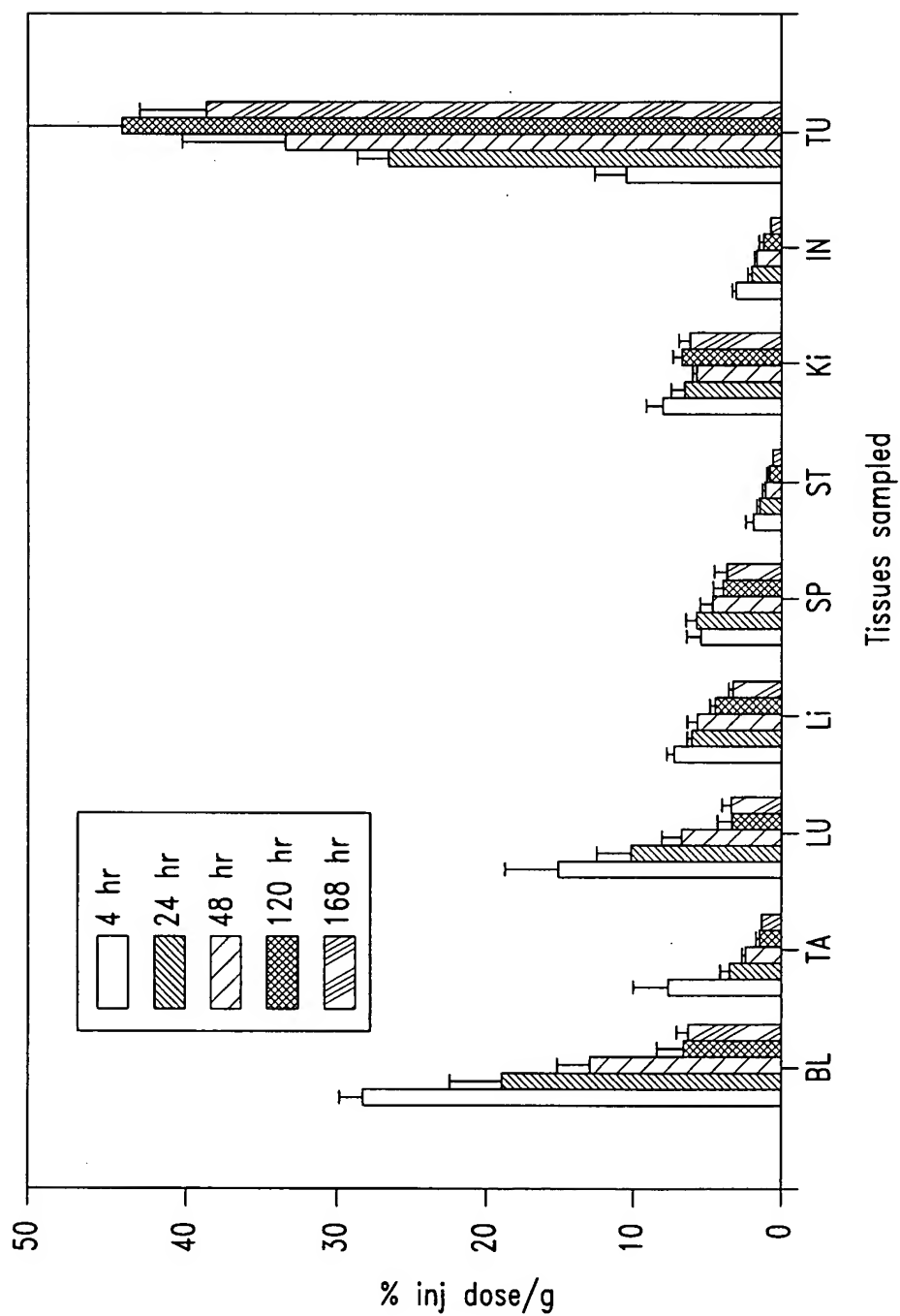
*Fig. 20*



*Fig. 21A*



*Fig. 21B*



*Fig. 22*



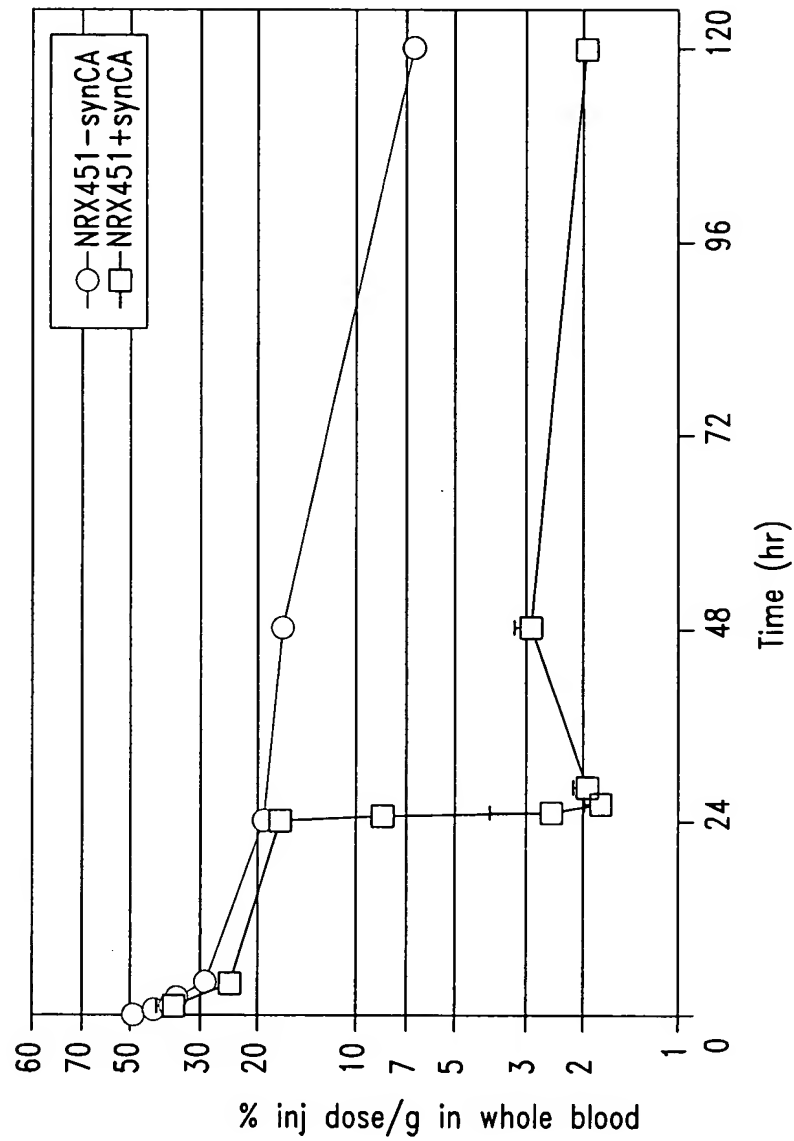


Fig. 23

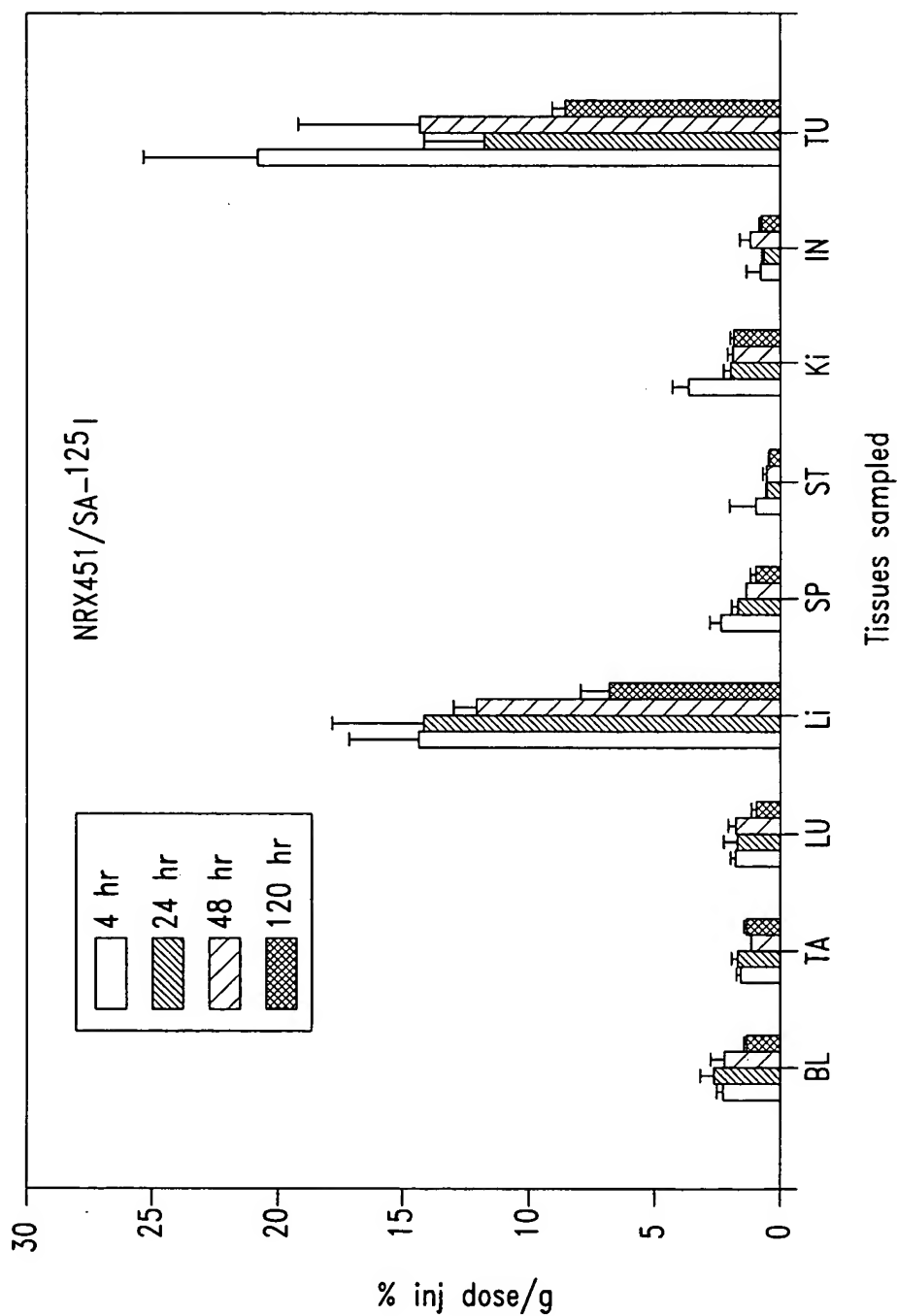
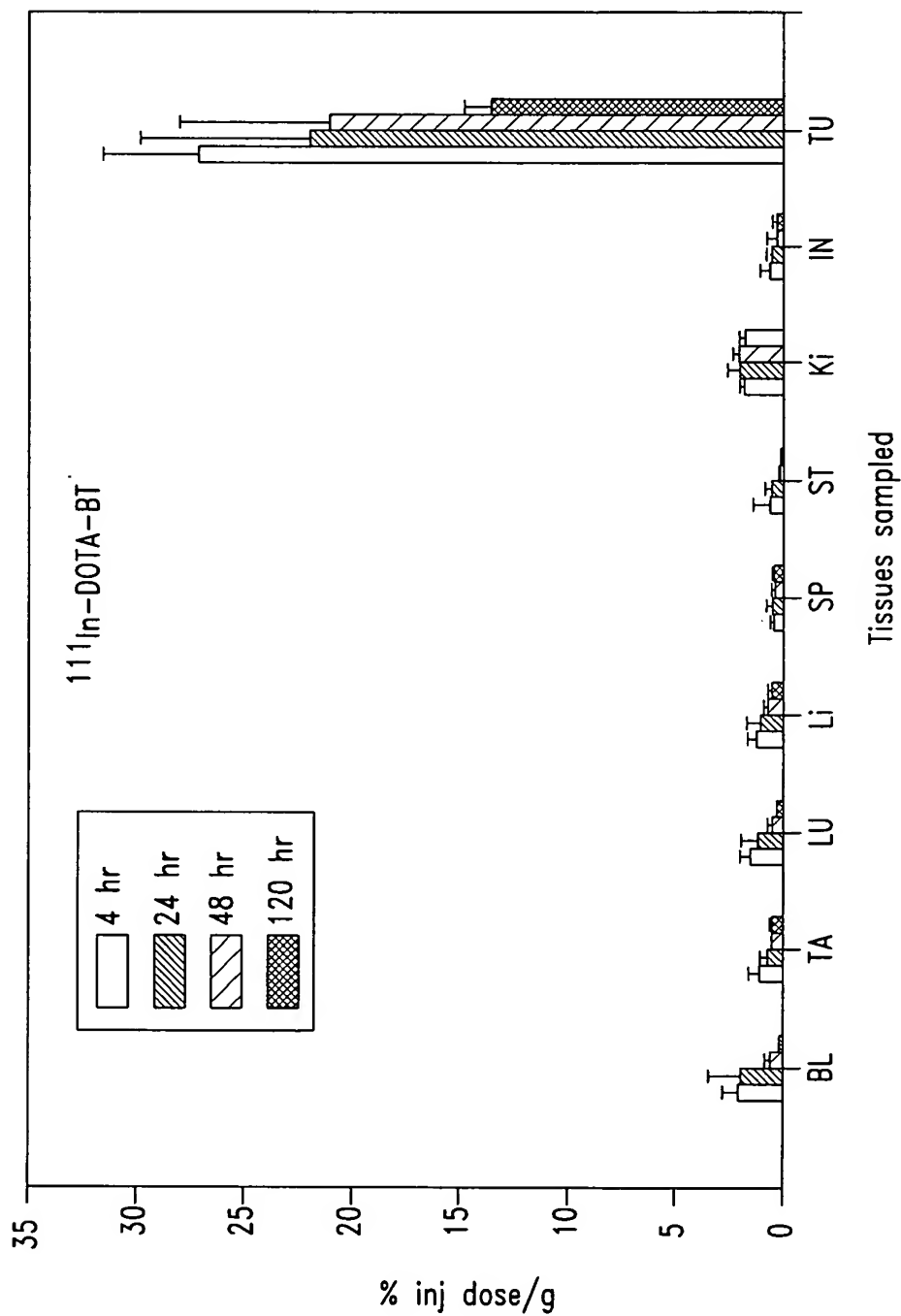


Fig. 24A



*Fig. 24B*